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Abstract

Whipple's disease is a rare systemic disorder classically presenting with weight loss, arthralgias, and diarrhea, which was first described in 1907. The causative bacterium *Tropheryma whipplei*, is a fastidious organism not growing on conventional media. Before the introduction of polymerase chain reaction (PCR)-based methods, the diagnostic gold standard was histological detection of diastase-resistant periodic acid Schiff (PAS)-positive macrophages or electron microscopy. As in the present case, contradictory results between the former and new diagnostic methods may obscure the correct diagnosis. We critically summarize the performance of the different diagnostic methods and discuss their impact on the clinical management of patients with suspected Whipple's disease.

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Introduction

Intestinal lipodystrophy was first recognized as a new disorder in 1907 by the pathologist *George Hoyt Whipple* [1]. The histological criteria for Whipple's disease as summarized in 1949 were periodic acid Schiff (PAS)-positive inclusions detectable in macrophages of the intestines and mesenteric lymph nodes [2]. In 1991, the bacterium of Whipple's disease was partially characterized at the molecular level by broad-range bacterial 16S rDNA PCR and sequencing [3]. Isolation of the bacterium was achieved in the late 1990s in long-term culture systems with interleukin-4-deactivated human primary macrophages [4] and fibroblasts [5] providing a basis for further characterization of the organism [6]. Since then the organism is officially named *Tropheryma whipplei*. It is a small, uniform, rod-shaped, gram-positive, not acid-fast bacterium measuring 0.2×1.5–2.0 μm in size [7, 8]. By transmission electron microscopy, the bacterial cell wall appears as a trilamellar structure. The analysis of its small (925 kb) single circular chromosome points to a host-restricted lifestyle and immune evasion as an important role in the pathogenesis of the chronic course of Whipple's disease [9]. The modern molecular-based techniques greatly improved the diagnostic methods to recognize Whipple's disease which is characterized by a great variation in clinical presentation [7, 8]. Untreated Whipple's disease has a chronic progressive and potentially fatal course due to cardiac or central nervous system failure, wasting syndrome or septic shock [7, 8, 10, 11]. More than 90% of the patients respond to antibiotic therapy, but about 5 to 30% relapse despite prolonged treatment [7, 8, 12, 13]. Difficulties occur for the clinicians when the results of the different methods are contradictory. Such conflicting results may lead to a false diagnosis and death of the patient, as the present case demonstrates.

Case Report

A 66-year-old man was admitted to the hospital because of recurrent fever, arthralgias, and exanthema. The patient had been well until 7 years earlier when polymyalgia rheumatica was diagnosed and was treated with prednisone and methotrexate. Two years before admission, intermittent episodes of fever with leukocytosis and elevated C-reactive protein (CRP) levels occurred, which were successfully treated with amoxicillin. On admission the patient complained of weight loss, irregular bowel movements with constipation and diarrhea, polyarthralgias, pain and stiffness of the proximal limbs, sicca symptoms, pleuritic pain, and a pale patchy rash. Laboratory analysis showed anemia (hemoglobin 11.8 g/dl), leukocytosis (22.8 X 109/l) with neutrophilia (97%) and lymphopenia (1.8%), and elevated inflammatory markers, i.e. blood sedimentation rate of 82 mm/h and CRP 76 mg/l. Cultures from blood, urine, stool, and knee joint fluid did not reveal

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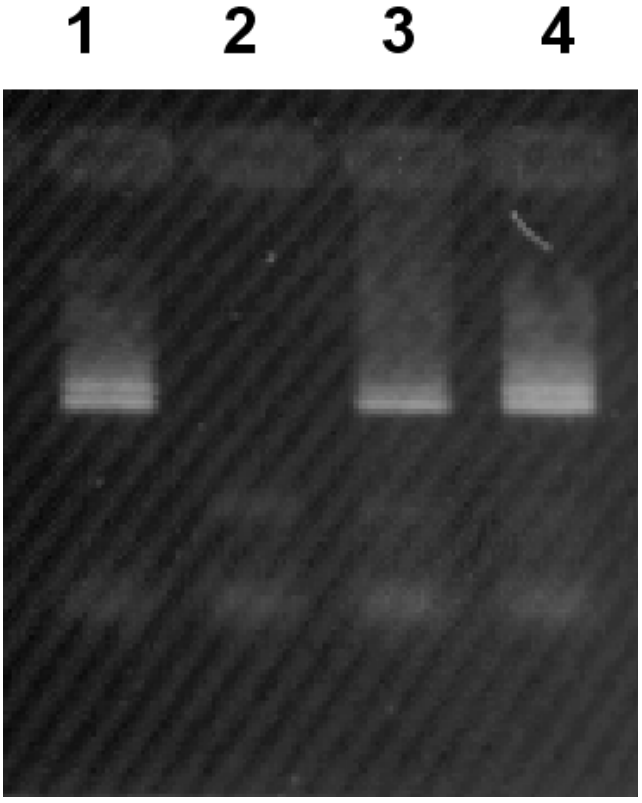

Figure 1. PCR from the knee joint fluid. Agarose gel electrophoresis of PCR products after semi-nested amplification using primer pairs TW1/TW2 (upper band, amplicon size 267bp) followed by TW4/TW2 (lower band, amplicon size 229bp). **Lane 1**, positive control with a constructed plasmid; **lane 2**, negative control with a strain of *Escherichia coli*, passing the whole procedure of DNA extraction and PCR reaction; **lane 3**, joint fluid of patient, undiluted DNA extract; **lane 4**, joint fluid of patient, DNA extract diluted 1:5. The relative intensities of bands in **lanes 3** and **4** indicate that the amplification was slightly inhibited when undiluted DNA extract was tested.

a causative pathogen. Serum protein electrophoresis, immunoglobulins, and serological tests for infections and autoantibodies were negative. Additional diagnostic procedures including bone marrow and skin biopsy, MR-angiography, echocardiography, positron emission tomography, and endoscopy were unremarkable. PCR from the knee joint fluid and a duodenal biopsy by semi-nested amplification using the primer pairs TW1/TW2 and TW4/TW2 were positive for *T. whipplei* (Figure 1). However, broad-spectrum bacterial PCR using a 16S rRNA gene fragment as well as the confirmation by another *T. whipplei*-specific PCR using a different technique [14] performed on the same specimens were negative. Further investigation by histological examination of duodenal biopsies did not reveal PAS-positive macrophages. Therefore, Whipple's disease was ruled out and a systemic inflammatory disorder of unknown origin was assumed. During the following 3 months, the patient was treated with indometacin and prednisone, but the clinical situation worsened and he died of multiorgan failure. Examination at autopsy revealed foamy macrophages filled with diastase-resistant PAS-positive particles in the lamina propria of the small and large intestines, the myo- and

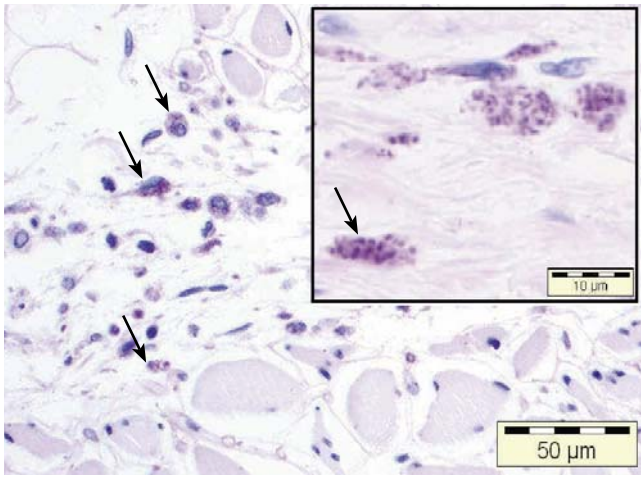

Figure 2. Pathological finding at autopsy: specimen of the myocardium stained with periodic acid Schiff (PAS) and the specific diastase PAS (inset). Microscopic examination demonstrates multiple macrophages engorged with PAS-positive material (arrows) between the myocytes and in the interfibrillar connective tissue.

pericardium (Figure 2), the skeletal muscles, the bone marrow, and the retroperitoneal soft tissue. Scanty PAS-positive granular inclusions were also detected in hippocampal ganglion cells, but not in the liver, spleen, and lymph nodes. The joints were not examined at autopsy. Reevaluation of the duodenal biopsies also showed a small number of PAS-positive macrophages. Based on these pathological findings a final postmortem diagnosis of Whipple's disease was made.

Discussion

Whipple's disease is a systemic infection that may involve any major organ system. The leading symptoms of weight loss, arthropathy, and diarrhea are not specific. Thus, the clinical suspicion has to be confirmed by further diagnostic tests on specimens obtained from the distal duodenum, the jejunum or the site of clinical manifestation such as heart valves, lymph nodes, synovial tissue and cerebrospinal fluid (CSF). Even stool specimens may be used to demonstrate the presence of DNA for *T. whipplei* [15]. The histological hallmark is the presence of foamy macrophages staining purple with diastase-resistant PAS, whereas PAS staining alone is not completely specific. PAS-positive macrophages are also found in patients with infections caused by *Mycobacterium avium-intracellulare*, *Rhodococcus equi*, *Bacillus cereus*, *Corynebacterium* sp., *Histoplasma capsulatum*, or other fungi. Some of the histopathological features of Whipple's disease are known also to occur in melanosis coli, histiocytosis, Crohn's disease, and Waldenström's macroglobulinemia [7, 8]. A further histological finding in lymphatic tissue, liver and the gastrointestinal tract associated with Whipple's disease are non-caseating, epitheloid-cell granulomas (sarcoid-like) [15]. Confusingly, the reactive macrophages present in these unspecific lesions are PAS-negative indicating that they do not contain *T. whipplei.*

In addition, *T. whipplei* can be identified by electron microscopy in tissue samples from infected organs due to its unusual and highly specific trilamellar cell wall. However, electron microscopy is not a convenient method for rapid clinical diagnosis and data comparing its performance with other diagnostic methods in Whipple's disease are not available. Therefore, electron microscopy is primarily used in questionable cases [7, 8].

Since the sequencing of the 16S rDNA gene and the description of specific primers for *T. whipplei*, gene amplification with PCR has been introduced as a diagnostic tool. However, clinicians have to be aware of several different factors influencing the performance of PCR. Native clinical specimens give better results as compared to formalin-fixation tissue due to partial degradation of the DNA [7]. Moreover, DNA extraction which is one of the crucial steps of all PCR techniques has to be adapted for particular clinical samples especially for those containing inhibitors of the Taq polymerase (e.g. feces) [7, 16]. Amplification with semi-nested and nested methods is associated with a higher risk for contamination, which can be reduced by using at least two independent PCR tests based on different target genes [8]. The sensitivity of these molecular tests also depends on the target gene and the length of the amplified fragments [7]. Specimens suitable for PCR are duodenal-biopsy tissue, lymph nodes, heart valves, vitreous humor, stool, and synovial or cerebrospinal fluid [8]. PCR may also be positive in samples from the sites of clinical manifestations of Whipple's disease, e.g. from a disc biopsy in a patient with spondylodiscitis or from joint fluid as in the present case [7, 12, 17]. However, it is currently impossible to detect *T. whipplei* DNA reproducibly from peripheral blood samples in patients with proven disease [7, 18]. On the other hand, *T. whipplei* was amplified from saliva, dental plaque, gastric juice, duodenal-biopsy samples, and feces in 4 to 35 % of healthy persons and patients without Whipple's disease [14, 16, 19–22], indicating that the diagnostic value of these specimens is limited in patients with a low clinical pretest probability.

Due to the rareness of Whipple's disease, quantitative comparative assessment of the different diagnostic methods is limited by the lack of studies directly addressing this question in a sufficient number of patients. Currently, there is no diagnostic gold standard conclusively defining Whipple's disease. Therefore, analyzing all published cases of Whipple's disease collected by *Dutly* and *Altwegg* [7], as well as data reported in more recent studies [14, 16, 19–23], we calculated the sensitivity and specificity of PCR and histology to detect Whipple's disease (Table 1). In gastrointestinal samples the sensitivity of PCR and histology were similar. In contrast, PCR techniques had a higher sensitivity than the presence of PAS-positive macrophages in histological evaluations of specimens from involved organs. The specificity of PCR was limited by false-positive results on saliva, dental plaque, and gastrointestinal samples in patients without Whipple's disease. As mentioned above, histological results showing the presence of macrophages with PAS-positive inclusions are not specific for Whipple's disease; however quantitative data on this issue are not available.

In conclusion, untreated Whipple's disease has a chronically progressive and potentially fatal course. However, most patients respond to antibiotic treatment with ceftriaxone, trimethoprim-sulfomethoxazole, or tetracycline resulting in rapid improvement of the clinical status and lasting remissions [7, 8, 12, 13]. Therefore, in cases of contradictory results between the former gold standard, PAS staining of duodenal biopsies, and recently introduced, highly sensitive PCR techniques, antibiotic treatment is warranted. In addition, critically reviewing the diagnostic results including meticulous reevaluation of all specimens and repeated sampling may help to find the correct diagnosis.

Table 1
Evaluation of histology and polymerase chain reaction for Whipple's disease.

Samples from	Gastrointestinal tract		Involved organs	
	Histology	PCR	Histology	PCR
Sensitivity (95% CI)	78% (71–85)[a]	84% (71–92)[b]	79% (64–90)[c]	100% (87–100)[d]
Specificity (95% CI)	NA	94% (92–95)[e]	NA	NA

Histology: detection of periodic acid Schiff (PAS)-positive macrophages; PCR: polymerase chain reaction with different primers and techniques; CI: confidence interval; NA: not available. Sensitivity and specificity including exact 95% binominal confidence intervals were calculated using the patients published in the references [14, 16, 19–23]. Patients redundantly described in more than one reference were counted only once. [a] Biopsies from the gastrointestinal tract were diagnostic in 123 of 157 patients with intestinal or extraintestinal manifestations of Whipple's disease. [b] PCR from gastrointestinal samples (biopsies, gastric juice, stool, saliva) was positive in 46 of 55 patients with intestinal or extraintestinal manifestations of Whipple's disease. [c] Biopsies from involved extraintestinal organs were diagnostic in 34 of 43 patients with Whipple's disease. [d] PCR from involved extraintestinal organs was positive in 27 of 27 patients with Whipple's disease. [e] PCR from gastrointestinal samples (biopsies, gastric juice, stool, saliva, dental plaque) was positive in 60 of 970 patients without Whipple's disease

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